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Assessment of VAV2 Expression Refines Prognostic Prediction in Adrenocortical Carcinoma

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1 CLINICAL RESEARCH ARTICLES

2 **Assessment of VAV2 expression refines
prognostic prediction**

3 **in adrenocortical carcinoma**

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32 **Short title:** VAV2 in adrenocortical carcinoma 33

. 34 **Précis:** We studied VAV2 expression in a large multicentric
cohort of adrenocortical

. 35 carcinoma cases and validated its role as a prognostic
marker.

. 36 **Keywords:** adrenocortical carcinoma, VAV2, Ki67, prognostic
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. 54 **Abstract**

. 55 **Background:** Adrenocortical carcinoma (ACC) is a rare
endocrine malignancy with overall
. 56 poor prognosis. The Ki67 labeling index (LI) has a major
prognostic role in localized ACC
. 57 after complete resection but its estimates may suffer from
considerable intra- and
. 58 interobserver variability. VAV2 overexpression induced by
increased SF-1 dosage is an
. 59 essential factor driving ACC tumor cell invasion.
. 60 **Objective:** To assess the prognostic role of VAV2 expression
in ACC by investigation of a
. 61 large cohort of patients.
. 62 **Design, Setting and Participants:** 171 ACC cases (157
primary tumors, 6 local
. 63 recurrences, 8 metastases) from seven ENS@T centers were
studied.
. 64 **Outcome Measurements:** H-scores were generated
quantifying VAV2 expression. VAV2
. 65 expression was divided into two categories, low (H-score <2)
and high (H-score ≥2). Ki67
. 66 LI retrieved from patients' pathological records was also
categorized into low (<20%) and

- . 67 high ($\geq 20\%$). Clinical and immunohistochemical markers were correlated with progression-
- . 68 free (PFS) and overall survival (OS).
- . 69 **Results:** VAV2 expression and Ki67 LI were significantly correlated with each other and
- . 70 with PFS and OS. Heterogeneity of VAV2 expression inside the same tumor was very low.
- . 71 Combined assessment of VAV2 expression and Ki67 LI allowed to improve patient
- . 72 stratification to low-risk and high-risk groups.
- . 73 **Conclusion:** Combined assessment of Ki67 LI and VAV2 expression improves prognostic
- . 74 prediction in ACC.

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. 79 **Introduction**

- . 80 Adrenocortical carcinoma (ACC) is a rare endocrine malignancy with overall poor
- . 81 prognosis, limited treatment options when progressed into metastatic stage and
- . 82 unsatisfactory response to polychemotherapeutic cytotoxic regimens (1, 2). Hence the
- . 83 most efficient method to eradicate the disease consists in complete surgical resection of
- . 84 the primary tumor. However, risk of recurrence is high even in this condition. Molecular

- . 85 studies have identified two subclasses of ACCs with aggressive (C1A) or indolent (C1B)
- . 86 clinical behavior, respectively (3-6). However, since molecular markers identified by those
- . 87 studies have not yet found entrance into clinical practice, it would be of particular
- . 88 importance to stratify patients with ACC into low-or high-risk groups to adequately monitor
- . 89 disease recurrence and assign them to appropriate therapeutic interventions. The
- . 90 histological Weiss score, which is commonly used as an established morphometric
- . 91 criterion for the differential diagnosis in adrenocortical tumors, has limited value as a
- . 92 prognostic indicator, especially in cases with borderline features (7, 8). Conversely, it was
- . 93 shown that a number of immunohistochemical markers have a prognostic value in ACC (9-
- . 94 18). Among those, the most widely used in clinical pathology reports is the Ki67 labeling
- . 95 index (LI), which is directly related to the proliferative activity of a given tissue (14-18). A
- . 96 study recently completed by the European Network for the Study of Adrenal Tumors
- . 97 (ENS@T) could indeed demonstrate that Ki67 LI has a major prognostic role in localized
- . 98 ACC after complete resection (18). However, Ki67 LI estimates suffer from considerable
- . 99 intra- and interobserver variability, as highlighted in a recent

study (19). New prognostic

. 100 markers are therefore needed to further refine prognostic classification of patients with

. 101 ACC as part of a multiparametric analysis.

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. 102 The transcription factor Steroidogenic Factor-1 has a pivotal role in regulating

. 103 adrenocortical cell proliferation and differentiation (20). Its overexpression is associated to

. 104 adrenocortical tumorigenesis through regulation of a specific set of SF-1 dosage-

. 105 dependent target genes (21, 22). One of these genes encodes VAV2, a guanine

. 106 nucleotide exchange factor (GEF) for small GTPases of the Rho family (23). We have

. 107 recently shown that VAV2 overexpression induced by an increased SF-1 dosage in ACC

. 108 is an essential factor driving tumor cell invasion (24). Herein, we present the results of a

. 109 large study involving ACC cases provided by seven European institutions aimed to assess

. 110 the prognostic value of VAV2 expression in ACC and to compare and integrate it with the

. 111 Ki67 LI.

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. 114 **Materials and Methods**

. 115 **Immunostaining on formalin-fixed, paraffin embedded ACC samples**

. 116 We analyzed a total of 171 adrenocortical tumor tissues from patients with ACC

. 117 provided by seven ENS@T centers (Italy 103, The Netherlands 42, France 20, Germany 6

. 118 samples). 145 samples were previously assembled in 7 tissue microarrays (TMA) with 2 or

. 119 3 cores per sample, interspersed with normal human liver, kidney and placenta tissues,

. 120 and 26 samples were available as full slides. Among the ACC samples, 157 samples

. 121 derived from primary tumors (male/female 59/98, average age \pm SD 48.7 \pm 15.2 years,

. 122 average tumor size \pm SD 11.2 \pm 5.4 cm; for patients' characteristics see Table S1), 6 from

. 123 local recurrences and 8 from distant metastases (liver and lung). The diagnosis of ACC

. 124 was made by established criteria based on clinical, biochemical and morphological data

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. 125 (25). All clinical data were collected through the ENS@T database (registry.ensat.org). All

. 126 patients gave informed consent and the study was approved by ethical committees from

. 127 all participating institutions. Immunohistochemical detection was performed in all samples

. 128 using an indirect immunoperoxidase technique after high temperature antigen retrieval in

. 129 0.01 M citrate buffer (pH 6.5) in a pressure-cooker for 13 minutes. The primary antibody

- . 130 was a rabbit monoclonal antibody against the VAV2 protein (clone EP1067Y, ab52640
- . 131 Abcam) diluted 1:250 in 25% AB serum in PBS and incubated 1 h at RT. Signal detection
- . 132 was performed with the Advance HRP detection system (Dako) and DAB chromogen
- . 133 according to the manufacturer's instructions. Nuclei were counterstained with Mayer's
- . 134 hematoxylin for 3 minutes. As negative control, universal rabbit negative control (Dako)
- . 135 was used. Immunostaining results were analyzed using a light microscope at high
- . 136 magnification. VAV2 staining intensity was evaluated independently by two investigators
- . 137 blinded to the clinical data (S.S. and I.S.). Cytoplasmic staining intensity was evaluated
- . 138 with a grading score of 0, 1, 2 or 3, corresponding to negative, weak, moderate and strong
- . 139 intensity, respectively. The proportion of positive tumor cells was calculated for each
- . 140 specimen and set up to be scored 0, 0.1, 0.5 or 1, if 0%, 1-9%, 10-49% or >50% of the
- . 141 tumor cells were positive for VAV2, respectively. A semi-quantitative H-score was then
- . 142 calculated by multiplying the staining intensity grade by the proportion score (12, 24). In all
- . 143 cases analyzed, the proportion of VAV2 positive cells was always >50%, so all intensity
- . 144 values were multiplied by a factor equal to 1 to yield the H-

score. The cut-off point to

- . 145 separate samples in high or low VAV2 expression was
between H-scores <2 and ≥ 2 . Ki67
- . 146 LI data assessed by the local pathologists in each expert
center were retrieved from the

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- . 147 ENS@T database. The Ki67 LI cut-off value used in this
study to separate low LI and high
- . 148 LI groups was 20%.

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. 150 **Statistical analysis**

- . 151 Correlation analyses were performed using a χ^2 test for
categorical variables. The
- . 152 inter-observer agreement for the scoring system was
evaluated using Cohen's kappa-
- . 153 coefficient and confirmed using Pearson's correlation
coefficient. As cutoff for strong
- . 154 agreement 0.81 was chosen for the kappa-coefficient and
0.75 for Pearson's coefficient
- . 155 (26). The comparison of clinical and histopathological
characteristics was performed on
- . 156 GraphPad Prism 6.0 software using non-parametric Mann-
Whitney test (for two groups)
- . 157 and Kruskal-Wallis test with Dunn's correction for multiple
testing (for more than two
- . 158 groups), as appropriate. A p value <0.05 was considered to
be statistically significant.
- . 159 Survival analysis for ACC patients was calculated as

described (24) using the Kaplan-

- . 160 Meier method and differences between groups were assessed with log-rank and Cox
- . 161 proportional hazards statistics, using the SPSS software package (version 23.0.0 for Mac),
- . 162 after adjustment for sex, age and tumor stage. Progression-free survival (PFS) was
- . 163 defined as time elapsed from primary resection of ACC to the first recurrence, loco-
- . 164 regional or systemic. Overall survival (OS) was defined as time elapsed from primary
- . 165 resection of ACC to disease-related death or last follow-up visit. In the group of patients
- . 166 with R0 resection, OS data were available for 100 (VAV2) and 105 (Ki67 LI) patients,
- . 167 respectively. 92 of those patients had both VAV2 and Ki67 LI OS data available. Viable
- . 168 cell data after VAV2 knockdown were analyzed by 1-way ANOVA with Dunnett's
- . 169 correction for multiple comparisons.

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. 171 **Results**

. 172 **VAV2 expression is a strong predictor of PFS and OS in ACC patients**

- . 173 Examples of different VAV2 expression patterns in ACC are shown in Fig. 1. A H-
- . 174 score was assigned to each sample, which took in

consideration both staining intensity

- . 175 and the percentage of cells stained by the anti-VAV2 antibody. The inter-observer
- . 176 agreement was very good with Cohen's kappa coefficient equal to 0.85 (95%CI: 0.72-
- . 177 0.89) and Pearson coefficient $r=0.90$ (95%CI:0.86-0.93), $p<0.001$. In contrast to Ki67
- . 178 staining, which is usually heterogeneous throughout a tumor, VAV2 expression was fairly
- . 179 equally distributed within a given tumor, with all samples presenting a percentage of
- . 180 stained cells $>50\%$. H-score heterogeneity among different TMA tissue cores belonging to
- . 181 the same tumors was limited, with a residual standard deviation $\sigma=0.14$ and an intra-class
- . 182 correlation coefficient $\alpha=0.95$ (95%CI: 0.92-0.97) (Fig. S1). The same homogenous
- . 183 distribution was also observed when whole tumor slides were analyzed (Fig. 1). VAV2
- . 184 expression in the tumor was strongly correlated to both PFS (Fig. 2A) and OS (Fig. 2B),
- . 185 confirming the results of our previous study performed on an independent smaller cohort
- . 186 of ACC patients (24). Patients with strong VAV2 expression had a 2.8-fold higher risk to
- . 187 experience a recurrence and 1.6-fold increased risk to die. No statistically significant
- . 188 difference existed for VAV2 expression in primary tumors and metastatic sites from the

. 189 same patients ($p=0.67$). The Ki67 LI was also a strong
predictor of PFS (Fig. 2C) and OS
. 190 (Fig. 2D), as reported in previous studies (14-18). Both VAV2
expression and Ki67 LI were
. 191 strongly correlated with OS even in patients with R0
resection (Fig. S2). VAV2 expression

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. 192 and Ki67 LI had a similar strong prognostic value for PFS
and OS both in univariate and in
. 193 multivariate analysis, taking into account patients' age, sex
and tumor stage (Table 1).

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. 195 **Combined assessment of VAV2 expression and Ki67 LI
improves prognostic power**

. 196 In general, a significant correlation existed between Ki67 LI
and VAV2 expression
. 197 in our ACC cohort (Fig. S3). A strong correlation also existed
when Ki67 LI and VAV2
. 198 expression were considered as categorical (low vs. high)
variables ($\chi^2 = 6.18$, $p=0.01$).
. 199 However, in several cases these two parameters were
dissociated with one value being
. 200 elevated and the other low in the same tumor. Remarkably,
in those patients PFS and OS
. 201 were intermediate between the high-risk (high VAV2
expression-high Ki67 LI) and the low-
. 202 risk groups (low VAV2 expression-low Ki67 LI) (Fig. 3A, B).
Merging the groups with high

. 203 VAV2-low Ki67 LI and low VAV2-high Ki67 LI and comparing
them to the high VAV2-high

. 204 Ki67 LI and low VAV2-low Ki67 LI groups identified three
classes of patients with very

. 205 different RFS (159.7 ± 23.2 , 90.3 ± 15.7 and 20.8 ± 5.8 months,
respectively) and OS

. 206 (203.7 ± 29.6 , 130.3 ± 29.6 and 41.6 ± 5.1 months, respectively)
(Fig. 3C, D). This type of

. 207 stratification maintained a strong prognostic value even in R0
patients (Fig. S4).

. 208 Remarkably, when considering the high-risk group apart from
all other patients with ACC,

. 209 a very strong correlation existed with OS in the whole cohort
(Fig. 4A) and with both PFS

. 210 and OS in R0 patients (Fig. 4B, C). Furthermore, isolated
high VAV2 expression or high

. 211 Ki67 LI showed a prediction value for worse PFS and OS that
was slightly lower compared

. 212 with the combination of both high VAV2 expression + high
Ki67 LI [PFS: 22 months,

. 213 HR=0.67 (VAV2) and 28 months, HR=0.66 (Ki67 LI) vs. 9
months for the combination; OS:

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. 214 66 months, HR=0.73 (VAV2) and 40 months, HR=0.82 (Ki67
LI) vs. 33 months for the

. 215 combination].

216

. 217 **Discussion**

- . 218 The prognosis of ACC patients is variable and poorly predictable. A recent large
- . 219 multicentric ENS@T study has shown that the Ki67 LI is the most powerful parameter
- . 220 predicting disease recurrence and survival in ACC patients after complete tumor resection
- . 221 (18). The Ki67 LI has been integrated with the combined evaluation of morphological
- . 222 parameters (number of mitoses/presence of necrosis) in the newly introduced Helsinki
- . 223 score, which reportedly is able to more accurately predict recurrence in ACC (8, 27).
- . 224 However, even if Ki67 LI assessment is routinely performed in diagnostic pathology
- . 225 laboratories for a large number of neoplastic disorders, its standardization and
- . 226 reproducibility have been questioned for many tumor types, including ACC (19). It is
- . 227 therefore important to identify other molecular markers that can complement the Ki67 LI to
- . 228 obtain a more accurate stratification of the risk of recurrence in patients with ACC. In this
- . 229 perspective, molecular prognostic indicators derived from genomic studies are very
- . 230 promising (3, 28, 29), but for routine implementation they suffer from the important
- . 231 drawback that, at least at the present state of technology, frozen tumoral material is
- . 232 required. On the other hand, prognostic value of circulating

markers of malignancy awaits

- . 233 validation in large cohorts of ACC patients (30-33).
- . 234 We have recently shown that VAV2 overexpression is an essential driver of cell
- . 235 invasion in conditions of increased SF-1 dosage through its GEF activity for the small
- . 236 GTPases Rac1 and Cdc42 (24). Those data directly link VAV2 with the potential

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- . 237 mechanism of malignancy consisting in increased cellular invasiveness. In the present
- . 238 study we extended the previous study to a large European cohort of patients with ACC
- . 239 and show that the tumor VAV2 H-score is significantly correlated to PFS and OS. The
- . 240 combined assessment of VAV2 expression and Ki67 LI improves patient risk stratification,
- . 241 with cases presenting high Ki67 LI but low VAV2 expression having significantly longer
- . 242 PFS and OS compared to patients with concordant high-risk parameters. In our study,
- . 243 VAV2 H-score assessment, which was mainly performed on TMA tissue cores, was
- . 244 associated to an excellent intratumoral reproducibility and is then in principle less prone to
- . 245 intra- and interobserver variability, although further work is needed to specifically address
- . 246 this question on an even larger number of cases. These results show that

- . 247 immunohistochemical assessment of VAV2 expression may usefully complement the
- . 248 measurement of the Ki67 LI for prognostic stratification of patients with ACC.

249 250

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PFS	Variables	Univariate analysis			Multivariate analysis		
		HR	95% CI	p	HR	95% CI	p
	age (n=113; n=99)	1.00	0.98-1.02	0.77	0.99	0.98-1.01	0.91
	sex						
	female (n=78; n=69)						
	male (n=35; n=30)	1.19	0.69-2.04	0.52	1.17	0.64-2.13	0.59
	Tumor stage						
	I (n=12; n=8)						
	II (n=66; n=61)	7.19	0.98-52.52	0.05	5.32	0.71-39.39	0.10
	III (n=23; n=19)	6.29	0.81-48.65	0.07	5.03	0.64-39.65	0.12
	IV (n=11; n=11)	27.51	3.40-222.11	0.002	14.65	1.74-122.96	0.01
	VAV2 expression						
	VAV2 low (H-score 0-1) (n=52; n=52)						
	VAV2 high(H-score 2-3) (n=48; n=47)	2.80	1.57-4.98	<0.001	2.83	1.54-5.21	0.001
	Ki67 LI						
	Ki67 low (<20%) (n=63; n=62)						
	Ki67 high (≥20%) (n=42; n=42)	2.77	1.58-4.86	<0.001	2.43	1.37-4.31	0.002
OS	Variables	Univariate analysis			Multivariate analysis		
		HR	95% CI	p	HR	95% CI	p
	age (n=156; n=74)	1.01	0.99-1.03	0.14	1.01	0.98-1.03	0.35
	sex						
	female (n=98; n=79)						
	male (n=58; n=45)	1.27	0.76-2.12	0.35	1.39	0.80-2.41	0.24
	Tumor stage						
	I (n=12; n=8)	3.71	0.50-27.60	0.2			
	II (n=72; n=65)	3.71	0.50-27.60	0.2	3.77	0.49-28.84	0.20
	III (n=35; n=29)	4.52	0.59-34.49	0.14	3.85	0.49-29.87	0.19
	IV (n=24; n=22)	19.07	2.52-144.30	0.004	13.74	1.77-106	0.01
	VAV2 expression						
	VAV2 low (H-score 0-1) (n=66; n=60)						
	VAV2 high (H-score 2-3) (n=76; n=64)	1.64	1.01-2.66	0.042	2.03	1.07-3.83	0.02
	Ki67 LI						
	Ki67 low (<20%) (n=77; n=72)						
	Ki67 high (≥20%) (n=68; n=60)	2.94	1.67-5.19	<0.001	2.31	1.24-4.30	0.008

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. 397 **Table 1. Analysis of parameters correlated with PFS and OS in univariate and**

. 398 **multivariate analysis.** Numbers of cases taken into account for univariate and

. 399 multivariate analysis, respectively, are indicated in parentheses for each variable.

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401 402 403

. 404 **Abbreviations:** CI, confidence interval; HR, hazard ratio; PFS, progression free-survival;

. 405 OS, overall survival.

Figure legends

Figure 1. Examples of various intensities of VAV2 staining in ACC specimens. H-score value is indicated for each image, respectively. Scale bar, 400 μ m (images in left column); 50 μ m (images in right column).

Figure 2. Correlation of VAV2 expression (H-score) and Ki67 LI with PFS and OS in our ACC series. (A) PFS in low VAV2 expression (H-score <2) group (green line) 127 ± 15.9 months; high VAV2 expression (H-score ≥ 2) group (red line) 25.7 ± 4.1 months. $p < 0.001$, Kaplan-Meier method. (B) OS in low VAV2 expression (H-score <2) group (green line) 180 ± 22 months; high VAV2 expression (H-score ≥ 2) group (red line) 87.4 ± 13 months. $p = 0.001$, Kaplan-Meier method. (C) PFS in low Ki67 LI ($<20\%$) group (green line) 137 ± 17.9 months; high Ki67 LI ($\geq 20\%$) group (red line) 68.5 ± 14.3 months. $p < 0.001$, Kaplan-Meier method. (D) OS in low Ki67 LI ($<20\%$) group (green line) 187.5 ± 22.9 months; high Ki67 LI ($\geq 20\%$) group (red line) 96.2 ± 17 months. $p = 0.001$, Kaplan-Meier method. The numbers of cases analyzed for each group are reported in parentheses.

Figure 3. Correlation of combined VAV2 expression (H-score) and Ki67 LI with PFS and OS in our ACC series. (A) PFS in low VAV2 expression (H-score <2)-low Ki67 LI ($<20\%$) group (green line) 159.7 ± 23.2 months; high VAV2 expression (H-score ≥ 2)-low Ki67 LI ($<20\%$) group (yellow line) 50.7 ± 8.4 months;

low VAV2 expression (H-score <2)-high Ki67 LI ($\geq 20\%$) group (pale green line) 96.6 ± 26.3 months; high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 20.8 ± 5.8 months. Compared to low VAV2-low Ki67 LI: high VAV2-low Ki67 LI HR=2.55 (1.09-5.97), $p = 0.030$; low VAV2-high Ki67 LI HR=2.46 (0.97-6.23), $p = 0.058$; high VAV2-high Ki67 LI HR=6.75 (2.97-15.31), $p < 0.001$; Kaplan-Meier method. (B) OS in low VAV2 expression (H-score <2)-low Ki67 LI ($<20\%$) group (green line) 203.7 ± 29.6 months; high VAV2

expression (H-score ≥ 2)-low Ki67 LI ($<20\%$) group (yellow line) 120.4 ± 20.5 months; low VAV2 expression (H-score < 2)-high Ki67 LI ($\geq 20\%$) group (pale green line) 126 ± 26.7 months; high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 41.6 ± 5.1 months. Compared to low VAV2-low Ki67 LI: high VAV2-low Ki67 LI HR=2.66 (1.08-6.52), $p=0.032$; low VAV2-high Ki67 LI HR=3.51 (1.38-8.91), $p=0.008$; high VAV2-high Ki67 LI HR=5.38 (2.33-12.40), $p<0.001$; Kaplan-Meier method. (C) PFS in low VAV2 expression (H-score < 2)-low Ki67 LI ($<20\%$) group (green line) 159.7 ± 23.2 months; high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 20.8 ± 5.8 months; all other patients with dissociated VAV2 expression-Ki67 LI group (grey line) 90.3 ± 15.7 months. Compared to low VAV2-low Ki67 LI: other HR=2.51 (1.17-5.39), $p=0.018$; high VAV2-high Ki67 LI HR=6.75 (2.97-15.31), $p<0.001$; Kaplan-Meier method. (D) OS in low VAV2 expression (H-score < 2)-low Ki67 LI ($<20\%$) group (green line) 203.7 ± 29.6 months; high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 41.6 ± 5.1 months; all other patients with dissociated VAV2 expression-Ki67 LI group (grey line) 130.3 ± 18.1 months. Compared to low VAV2-low Ki67 LI: other

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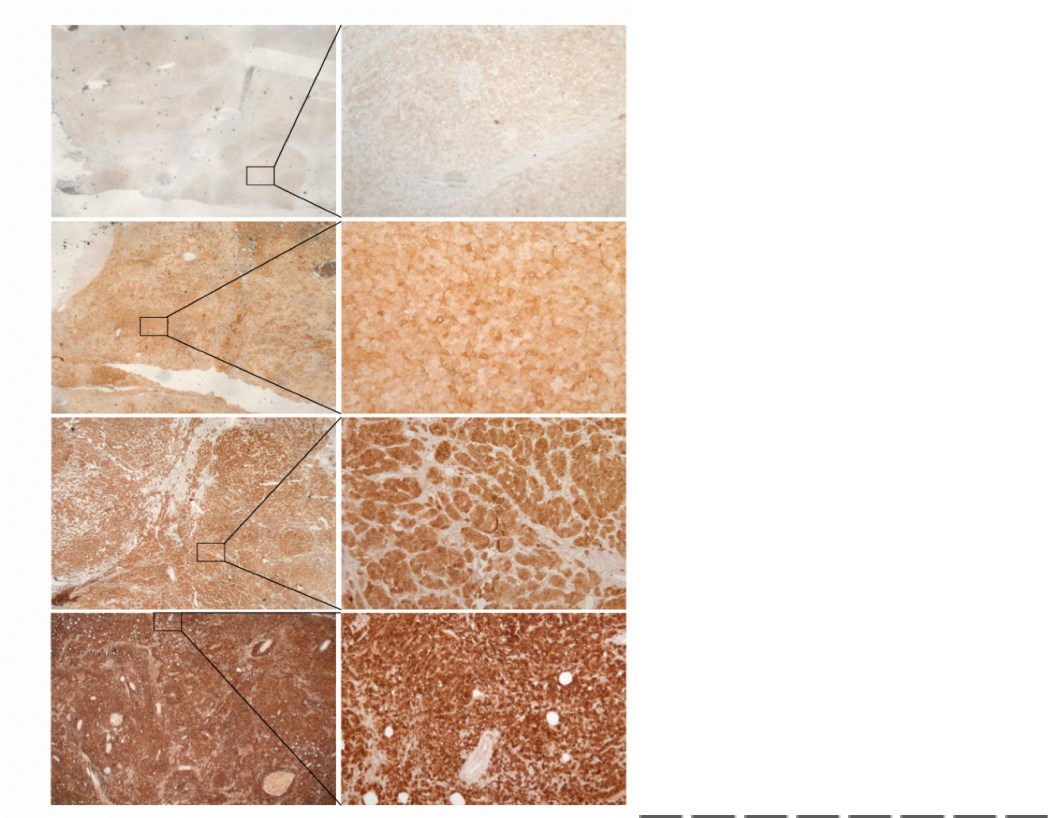
HR=2.99 (1.32-6.73), $p=0.008$; high VAV2-high Ki67 LI, HR=5.38 (2.33-12.40), $p<0.001$; Kaplan-Meier method. The numbers of cases analyzed for each group are reported in parentheses.

Figure 4. Prognosis of high-risk (high VAV2 expression-high Ki67 LI) vs. other ACC patients. (A) OS in the whole cohort of ACC patients for the high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 41.5 ± 5 months; all other patients (green line) 175.5 ± 19.8 months. $p<0.001$, Kaplan-Meier method. (B) PFS in R0 patients for the high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 20.8 ± 5.8 months; all other patients (green line) 127.3 ± 15.7 months. $p<0.001$, Kaplan-Meier method. (C) OS in R0 patients for the high VAV2 expression (H-score ≥ 2)-high Ki67 LI ($\geq 20\%$) group (red line) 47.5 ± 6 months; all other patients (green line) 194.8 ± 21.7 months. $p=0.005$, Kaplan-Meier method. The numbers of cases analyzed for each group are

reported in parentheses.

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Figure 1



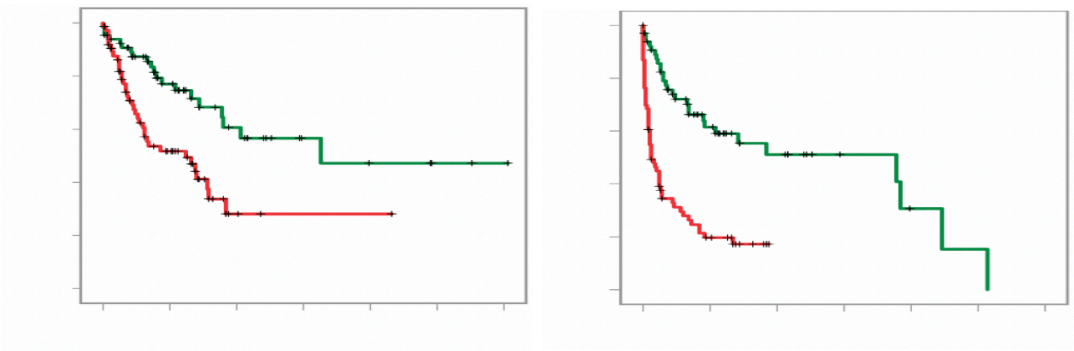
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H-score=3 H-score=2 H-score=1 H-score=0

Figure 2

AB 100

100 80



80 60 40 20

C 100

80 60 40 20

low VAV2 (n=52) 60 p<0.001 40

low VAV2 (n=66)

p=0.001

high VAV2 (n=76)

50 100 150 200 250 300 Time (months)

low Ki67 LI (n=77)

p=0.001

high Ki67 LI (n=68)

50 100 150 200 250 300 Time (months)

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high VAV2 (n=48)

50 100 150 200 250 300 Time (months) D

low Ki67 LI (n=63)

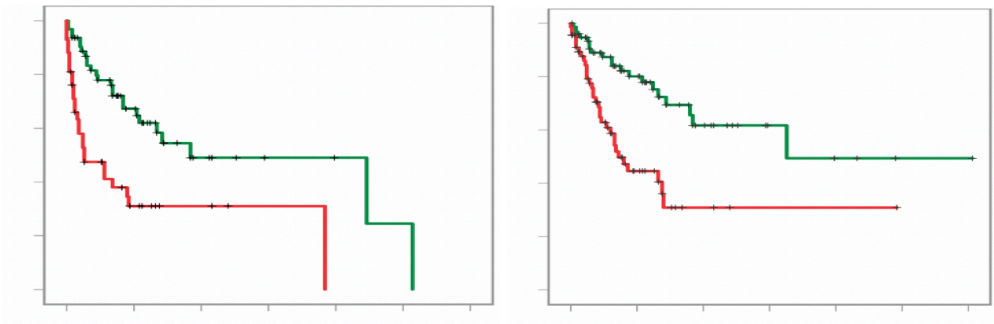
p<0.001

high Ki67 LI (n=42)

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0 50 100 150 200 250 300 Time (months)

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23

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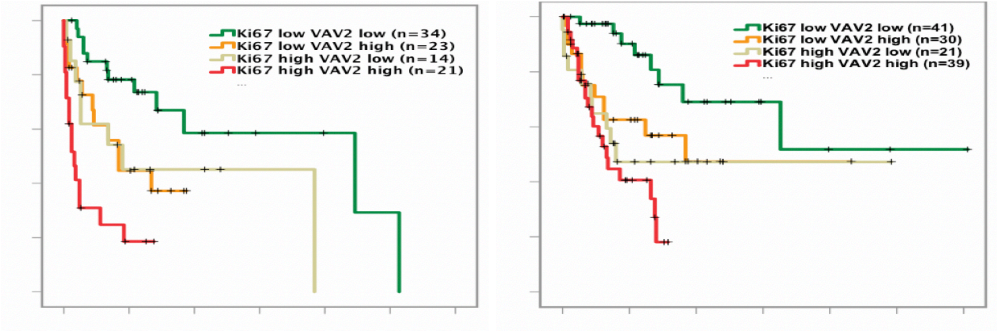
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Progression-free survival (%) Progression-free survival (%)

Overall survival (%) Overall survival (%)

Figure 3 AB



C

100 80 60 40 20

100 80 60 40 20

100 80 60 p=0.058 40 20 00

p=0.032

p<0.001

p=0.008

0

p=0.030 p<0.001

VAV2 low-Ki67 LI low(=34) VAV2 high-Ki67 LI low (=23) VAV2 low-Ki67 LI high (=14) VAV2 high-Ki67 LI high (=21)

50 100 150 200 250 300 Time (months) D

VAV2 low-Ki67 LI low (n=34)

other (n=37)

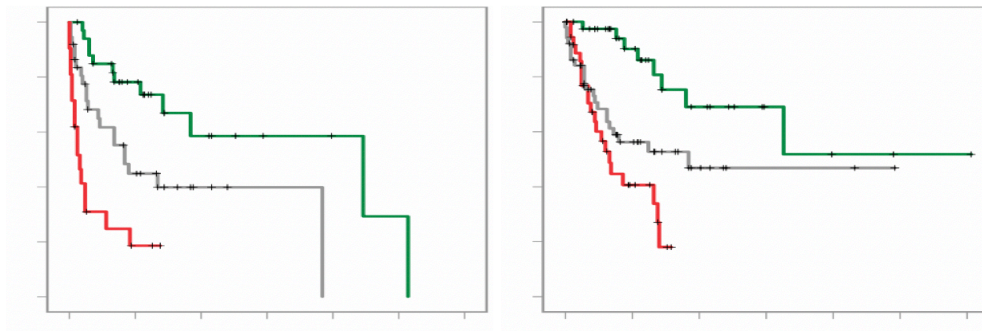
p=0.018

p<0.001

VAV2 high-Ki67 LI high (n=21)

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50 100 150 200 250 300 Time (months)



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0 50 100 150 200 250 300 Time (months)

0 50 100 150 200 250 300 Time (months)

24

100

80

60

40

20

VAV2 low-Ki67 LI low (n=41)

p<0.001

VAV2 high-Ki67 LI high (n=39)

other (n=51)

p=0.008

VAV2 low-Ki67 LI low (n=41) VAV2 high-Ki67 LI low (n=30) VAV2 low-Ki67 LI high (n=21) VAV2 high-Ki67 LI high (n=39)

Progression-free survival (%) Progression-free survival (%)

Overall survival (%) Overall survival (%)

Figure 4 A

100

80

60

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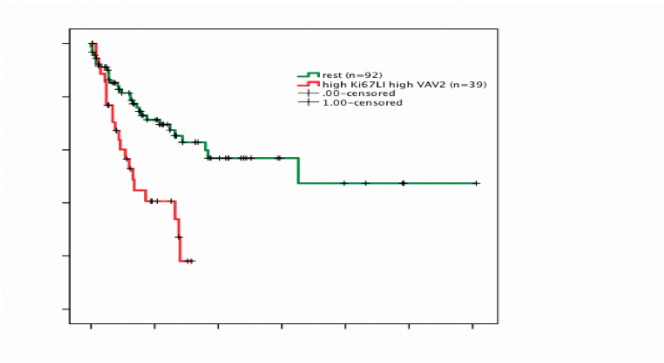
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100 80 60 40 20 0

other (n=92)

p<0.001

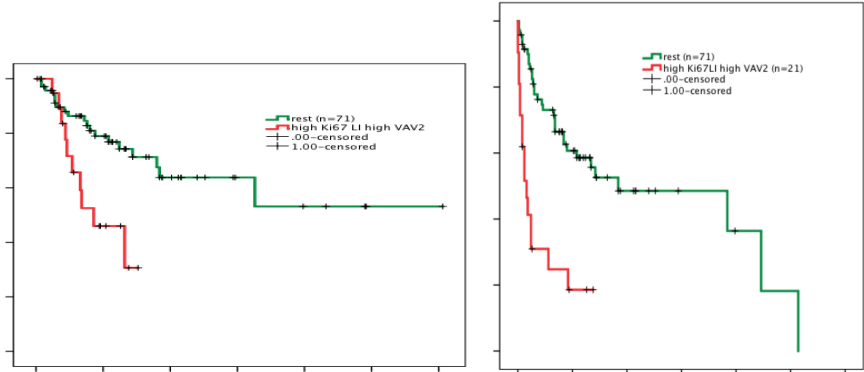


B

0 50 100 150 200 250 300 Time (months)

C

VAV2 high-Ki67 LI high (n=39)



other (n=71)

VAV2 high-Ki67 LI high (n=21)

p<0.001

100 80 60 40 20 0

other (n=71)

p=0.005

0 50 100 150 200 250 300 Time (months)

0 50 100 150 200 250 300 Time (months)

25

VAV2 high-Ki67 LI high (n=21)

Progression-free survival (%) Overall survival (%)

Overall survival (%)

